ously determine whether MAVS is involved in TLR3 signaling.

Although much work is required to fully describe the MAVS-dependent signaling pathways, the identification of MAVS and its mitochondrial localization is a major advance in our understanding of the intracellular detection of viral infection. The fact that mitochondria may be important in innate immunity hints at the possibility of a direct link between viral infection and the regulation of apoptosis. In fact, preliminary studies by Seth et al. show that knockdown of *MAVS* gene expression by small interfering RNAs leads to an increase in apoptosis. Thus, MAVS could protect cells from apoptosis during the early stages of viral infection, maximizing the production of cytokines from the infected cell. In any case, these exciting new findings highlight the central part played by mitochondria in balancing the host immune response and programmed cell death response to virus and possibly to other pathogens.

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Note Added in Proof

While this preview was in press, a related article appeared online (Kawai et al., 2005, Nat. Immunol., doi: 10.1038/ni1243). This article also reports the identification of MAVS (which the authors named IPS-1). Consistent with the studies featured here, IPS-1/MAVS is required for RIG-1 and Mda5-dependent induction of *IFN-β* gene expression. In addition, IPS-1/MAVS binds to RIG-1 and Mda5 as well as to both RIP1 and FADD. Finally, overexpression of dominant-negative FADD blocks IPS-1/MAVS-dependent expression of an NF-κB reporter gene, thus providing evidence that RIP1 and FADD mediate the activation of NF-κB through the RIG-1 pathway.

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"Bound" to Work: The Free Hormone Hypothesis Revisited

Megalin is a member of the low-density lipoprotein receptor-related protein (LRP) family. The plasma membrane-anchored LRPs serve as receptors for a wide variety of extracellular ligands, promoting their entry into cells by endocytosis of the receptor-ligand complex. In this issue of *Cell*, Hammes et al. (2005) show that resistance (insensitivity) to sex steroid hormones is encountered in animals lacking megalin. These data provide important insights into an endocytic mechanism for the uptake of sex steroids by mammalian cells.

The first hint that megalin, the largest member of the LRP family (Nykjaer and Willnow, 2002), may be important in maintaining the endocrine (hormone) balance of mammals came from the work of Nykjaer and colleagues (Nykjaer et al., 1999). They showed that mice lacking megalin developed vitamin D deficiency and as a result bone disease (vitamin D is required for maintaining healthy bones). This defect was due to the inability of the megalin-deficient luminal membrane of the proximal renal tubular epithelial cell to recognize and internalize the vitamin D precursor bound to its binding protein. This internalization abnormality then led to failed delivery of the vitamin D precursor to the mitochondrial enzyme that converts it to the active hormone, 1,25 dihydroxyvitamin D.

Hammes et al. (2005) now report that the vitamin D hormone pathway is not the only classical steroid hormone pathway that is dependent, at least in part, on megalin. They provide convincing evidence for a pathway for sex steroid uptake in which a complex of the serum sex hormone with its binding protein, the serum sex hormone binding globulin (SHBG), becomes internalized by interacting with megalin. SHBG is equivalent to the serum vitamin D binding protein for circulating androgens and estrogens. The presence of megalin appears to be crucial for optimal internalization of sex steroid hormones and for access of the hormone to its cognate intracellular receptor in vitro. This appears to be true in vivo, at least temporally, as female mice that lack megalin show abnormalities in vaginal opening in response to estrogen, and megalin-deficient male mice exhibit defects in testicular descent in response to androgens. Both conditions are specific sex steroid-dependent events in the postnatal development of rodent genitalia. Simply increasing the amount of androgen in pregnant mice did not rescue the embryonic genital defects, indicating that free hormone was not able to compensate for the lack of megalin. However, the fact that megalin-deficient animals are not phenocopies of mice lacking androgen or estrogen receptors suggests that there must be megalin-independent pathways for the entry of sex steroids into target cells expressing the appropriate hormone receptor.

The classic dogma regarding sex steroid hormone entry into target cells is the "free" hormone hypothesis

(see Figure 1, upper panel). This hypothesis states that, by virtue of its small size and lipid solubility, only the "free" steroid hormone, which has escaped the grasp of its circulating serum binding protein, can traverse the plasma membrane of target cells. Once inside the cell, the steroid hormone must locate the intracellular protein with which it needs to interact. For example, the target protein for testosterone may be the androgen receptor, or it may be the enzyme 5α-reductase for which testosterone is the preferred substrate and dihydrotestosterone the product. The inability to efficiently deliver androgen to its cognate receptor or testosterone to the 5α-reductase will result in incomplete androgenization of the host and a phenotype not dissimilar from that exhibited by male mice deficient in megalin (Griffin et al., 1982). However, as megalin-deficient animals are not completely resistant to androgens or estrogens, this suggests that either "free" hormone is somehow able to find its way to the androgen receptor or estrogen receptor, or that there exist other plasma-membrane receptors that promote the internalization of the steroid hormone. It is also possible, as suggested by Hammes et al. (2005) and others (reviewed by Mendel, 1989), that both entry mechanisms ("free" and "protein bound") coexist in the same cell.

One could envisage three steps in the protein bound entry mediated by megalin of estrogen and androgen (either testosterone or dihydrotestosterone) into target cells (see Figure 1, bottom panel). In the first step, the circulating SHBG protein bearing its sex steroid cargo interacts with megalin embedded in clathrin-coated pits (Nagai et al., 2005) in the plasma membrane. The next step is the formation of an endocytic vesicle, which envelops the binding protein and its cargo bound to megalin. Finally, there is dissociation of the binding protein from its steroid ligand inside the vesicle, presumably through acidification of the vesicle lumen (Gekle, 2005). If, as postulated by Hammes et al. (2005), protein bound androgens and estrogens can enter the cell by megalin-mediated endocytosis, how is it that they escape the confines of the endocytic vesicle to reach their intracellular destination? The "free" hormone hypothesis holds that once dissociated from the binding protein, the steroid diffuses through the vesicular membrane until it encounters its intracellular binding targets. Alternatively, as is the case with low-density lipoprotein (LDL)-cholesterol (Soccio and Breslow, 2004) internalization, there is mounting evidence suggesting the existence of trafficking intermediates (chaperones) for the delivery of small, lipid-soluble, vitamin D sterol and gonadal steroid hormones to specific intracellular proteins, including receptors and metabolizing enzymes (Adams et al., 2004). It is also possible that directed vesicular transport of the intact complex containing megalin bound to the sex steroid and its binding protein (that results from targeted docking of the cytoplasmically exposed, carboxyl terminus of the megalin molecule) is responsible for moving the steroid to such destinations (R. Chun and J.S.A., unpublished data).

Regardless of the mechanism, the Hammes et al. study and previous work force us to re-address an issue of substantial practical importance in the field of reproductive hormone biology. The issue is do measurements of the "free" (protein unbound) fraction of

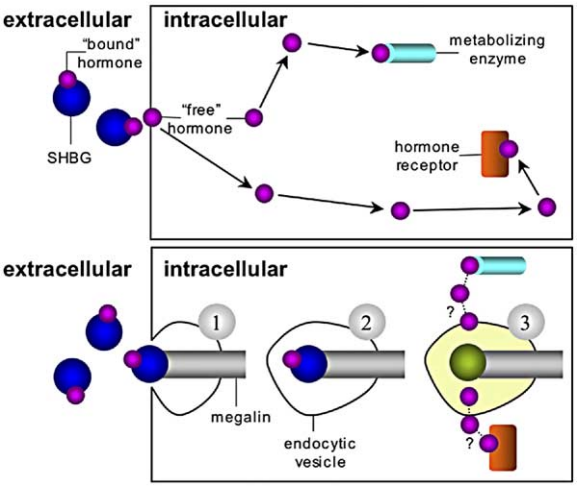

Figure 1. Possible Pathways for the Uptake of Sex Steroids by Target Cells that Contain Intracellular Steroid Receptors

(Upper panel) The "free" hormone mode of entry. In this model, steroids (pink) that are not bound to their cognate binding protein (SHBG, in the case of sex hormones, dark blue) diffuse across the plasma membrane and then interact with their intracellular protein targets: metabolizing enzymes (turquoise) or hormone receptors (orange). (Lower panel) The "bound" megalin-dependent mode of steroid hormone entry (Hammes et al., 2005). SHBG and its sex steroid hormone cargo bind to megalin (gray rod) concentrated in clathrin-coated pits in the plasma membrane (step 1) (clathrin is not shown). The hormone-SHBG-megalin complex then is internalized in endocytic vesicles (step 2). Megalin-mediated endocytosis is followed by release of the hormone cargo from its binding protein (dark green), which may occur during vesicle acidification (step 3, pale green). How the hormone, regardless of its route of internalization, is localized to specific intracellular protein targets including cognate sex steroid receptor proteins or metabolizing enzymes (such as, 5α-reductase or aromatase) remains unknown.

total hormone present in the plasma or serum of the host constitute the biologically relevant form of the steroid that interacts with its intracellular receptor and regulates hormone-directed transcription? Current estimates are that 30% of American males in the 60- to 70-year-old age group and 70% of those in the 70- to 80-year-old age group have low serum bioavailable or "free'" testosterone levels (Hijazi and Cunningham, 2005). If not at risk for androgen-promoted prostate or cardiovascular disease, should we consider male hormone replacement in such men a treatment that enhances muscle mass, strength, mood, and libido and treats osteopenia (low bone mass), osteoporosis, and erectile dysfunction, if their "free" testosterone is low? Although this field requires substantial further research, the results that Hammes et al. present here suggest that we may need to re-examine clinical decisions based on the extracellular concentration of "free" hormone.

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